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Characterization of the antioxidant system during the vegetative development of pea plants

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Abstract

The antioxidative system was studied during the development of pea plants. The reduced glutathione (GSH) content was higher in shoots than in roots, but a greater redox state of glutathione existed in roots compared with shoots, at least after 7 d of growth. The 3-d-old seedlings showed the highest content of oxidised ascorbate (DHA), which correlated with the ascorbate oxidase (AAO) activity. Also, the roots exhibited higher DHA content than shoots, correlated with their higher AAO activity. The activities of antioxidant enzymes were much higher in shoots than in roots. Ascorbate peroxidase (APX) activity decreased during the progression of growth in both shoots and roots, whereas peroxidase (POX) activity strongly increased in roots, reflecting a correlation between POX activity and the enhancement of growth. Catalase activity from shoots reached values nearly 3 or 4-fold higher than in roots. The monodehydroascorbate reductase (MDHAR) activity was higher in young seedlings than in more mature tissues, and in roots a decrease in MDHAR was noticed at the 11th day. No dehydroascorbate reductase (DHAR) was detected in roots from the pea plants and DHAR values detected in seedlings and in shoots were much lower than those of MDHAR. In shoots, GR decreased with the progression of growth, whereas in roots an increase was seen on the 9th and 11th days. Finally, superoxide dismutase (SOD) activity increased in shoots during the progression of growth, but specific SOD activity was higher in roots than in shoots.

Additional key words: ascorbate, ascorbate-glutathione cycle, ascorbate peroxidase, catalase, glutathione, glutathione reductase, peroxidase, *Pisum sativum* L., redox state, superoxide dismutase.

Introduction

The regulation of the cellular redox state is a crucial factor in plant development and response to environmental stimuli (De Pinto and De Gara 2004). There is increasing evidence that ascorbate (ASC) is involved directly in processes regulating cell wall expansion, although the exact mechanisms remain unclear. The redox status of apoplastic ASC and the balance between ASC regeneration and H_2O_2 formation will influence the degree of lignification and cross-linking of cell wall components, either directly, by scavenging of radicals, or indirectly, by inhibiting activities of peroxidases (POXs) (Davey *et al*. 2002). The growth rate of lupin seedlings increased when they were grown in a hydroponic medium containing ascorbate or its precursor galactono-1,4-lactone. On the other hand, seedling size is reduced remarkably when the endogenous ASC content is decreased by treating seedlings with lycorine, an inhibitor of ASC biosynthesis (Arrigoni *et al*. 1997). A direct relationship between ascorbate availability and plant growth has been demonstrated using the *vtc1 Arabidopsis* mutant. This mutant, having only 30 % of the wild-type ascorbate content, grows more slowly and flowers later than the wild-type (Veljovic-Jovanovic *et al*. 2001). Not only does ASC availability *per se* alter plant growth,

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Abbreviations: AAO - ascorbate oxidase; ASC - ascorbate; reduced form - ASC-GSH cycle - ascorbate-glutathione cycle; APX - ascorbate peroxidase; BSO - *L*-butathione-[S,R]-sulfoximine; DHA - dehydroascorbate; DHAR - dehydroascorbate reductase; GR - glutathione reductase; GSH - glutathione, reduced form; GSSG - glutathione, oxidised form; MDHA - monodehydroascorbate; MDHAR - monodehydroascorbate reductase; $O_2^{\cdot -}$ - superoxide radical; POX - peroxidase; ROS - reactive oxygen species; SOD - superoxide dismutase.

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but in addition all enzymes of ASC-metabolism are involved in the control of plant development (De Pinto and De Gara 2004). In etiolated pea plants, the ascorbate redox enzymes changed in different ways during cellular differentiation. A decrease in the ASC content, and ascorbate peroxidase (APX) and monodehydroascorbate reductase (MDHAR) activities occurred from meristematic to differentiated cells, whereas ascorbate oxidase and dehydroascorbate reductase (DHAR) activities increased (De Pinto and De Gara 2004).

The most dramatic changes in ASC metabolism characterising plant development probably occur during germination of the so-called orthodox seeds (De Gara 2003). A rapid rise in ASC content and APX activity during the first stages of germination is a common strategy developed in orthodox seeds of herbaceous plants to cope with the increased generation of reactive oxygen species (ROS) (Cakmak *et al.* 1993, De Gara *et al.* 1997, Garnczarska and Wojtyla 2008).

Ascorbate oxidase (AAO), another enzyme involved in ASC metabolism, plays a role in cell elongation because of its extracellular localisation and high activity in rapidly-expanding tissues (Esaka *et al.* 1992). AAO is an apoplastic enzyme, which supports the idea that changes in apoplastic ascorbate metabolism are crucial, not only as a front line of defence against environmental changes, but even for plant development (Pignocchi *et al.* 2003). It has been suggested that AAO increases the rate of cell elongation by generating monodehydroascorbate (MDHA) and dehydroascorbate (DHA) (De Pinto and De Gara 2004). The implication of AAO in plant growth has been investigated in tobacco plants. The overexpression of an AAO transgene in the sense orientation produced a biomass accumulation greater than that of plants expressing the AAO transgene in the antisense orientation (Pignocchi *et al.* 2003).

A very well-known function of POXs in plant growth concerns the cross-linking of cell wall components and the polymerisation of lignin and suberin monomers at the expense of H_2O_2, together with an important role in detoxification processes (Pomar *et al.* 2002).

Another antioxidants involved in ROS detoxification is reduced glutathione (GSH). Both ASC and GSH take part in ASC-GSH cycle, in which APX is directly involved in H_2O_2 elimination (Noctor and Foyer 1998) and MDHAR, DHAR and glutathione reductase (GR) contribute to maintaining high contents of ASC and GSH and avoiding the accumulation of DHA, a product of ASC oxidation that can interfere negatively with plant growth (Arrigoni *et al.* 1997, Tommasi *et al.* 2001). GSH regulation is important in a number of key processes associated with plant growth and development (Maughan and Foyer 2006). The relatively high content of GSH in root tips, where actively-dividing cells are found, suggests that growing root tissues have a requirement for GSH (Maughan and Foyer 2006). The GSH/GSSG ratio clearly influences the cell-cycle progression. A high GSH/GSSG ratio is essential for cell division and proliferation during the initial stages of embryo development, but later in development the redox status of the glutathione pool decreases (Belmonte *et al.* 2005). Studies using a GSH-deficient mutant or *L*-butathione-[*S*,*R*]-sulfoximine (BSO; an inhibitor of GSH biosynthesis) treatments suggest that GSH plays a key role in promoting signals which permit cell-cycle progression in roots and hence root growth (Vernoux *et al.* 2000, Maughan and Foyer 2006).

In recent years, ROS have emerged as important regulators of plant growth, development and differentiation. Several reports have suggested a link between ROS and plant developmental physiology. Treatments with low H_2O_2 doses induced seed germination and plant growth (Korystov and Narimanov 1997), whereas $OH^\bullet$ radical scavengers inhibited root and shoot elongation, suggesting that ROS are required for growth generally as well as for root gravitropism (Schopfer 2001).

The aim of this work was to analyse the antioxidant metabolism during the vegetative development following pea seed imbibition and germination. We analysed the antioxidant mechanisms involved in the ASC-GSH cycle (APX, MDHAR, DHAR and GR enzymes and the ascorbate and glutathione contents) as well as other antioxidant enzymes such as POX, superoxide dismutase (SOD) and catalase (CAT). The level of AAO activity was also analysed, bearing in mind its reported relationship with plant growth and development.

Materials and methods

Pea (*Pisum sativum* L., cv. Alaska) seeds were surface-sterilised (10 %, v/v, sodium hypochlorite for 2 min), germinated and grown in *Vermiculite*. Germination was carried out in the dark at 25 ºC. After 3 d, pea seedlings were grown in a growth chamber (day 0) at day/night temperature of 25/18 ºC, 80 % relative humidity and a 16-h photoperiod, at an irradiance of 200 µmol m^{-2} s^{-1}. For the assays, vigorous 3-d-old seedlings and 7, 9 and 11-d-old plants were selected.

We used the complete seedlings for analyses, while the plants were divided into shoots and roots. All operations were performed at 0 - 4 ºC. Samples (1.5 - 2 g fresh mass) were homogenised with an extraction medium containing 50 mM Tris-acetate buffer (pH 6.0), 0.1 mM EDTA, 2 mM cysteine and 0.2 % (v/v) *Triton X-100*. For the APX activity, 20 mM sodium ascorbate was added to the extraction buffer. The extracts were filtered through two layers of nylon cloth and centrifuged at 10 000 *g* for 15 min, at 4 ºC. The supernatant fraction was filtered on *Sephadex G-25 NAP* columns equilibrated with the same buffer as used for the homogenisation. For the APX activity, 2 mM sodium ascorbate was added to the equilibration buffer.

The activities of the ASC-GSH cycle enzymes, POX, CAT and SOD were assayed as described in Hernández *et al.* (2006a,b). AAO was analysed by monitoring the oxidation of ASC at 290 nm (Takahama and Oniki 1994). The glutathione and ascorbate contents were measured according to Griffith (1985) and Law *et al.* (1983), respectively. Protein was estimated according to Bradford (1976).

The changes of enzymatic and non-enzymatic antioxidants as well as plant growth and protein contents during development were tested by a one-way *ANOVA*, and comparisons among means were realized using Tukey's test calculated at $P < 0.05$. Correlation analyses were carried out by calculating the Pearson correlation coefficients. Statistical procedures were carried out with the software package *SPSS 15.0* for *Windows* (*SPPS Inc.*, Chicago, IL, USA).

Results

During plant development, the fresh mass of the whole pea plants had increased by nearly 2.5-fold at 7 d of growth in relation to 3-d-old seedlings (Table 1), and had increased by up to 4-fold at 11 d of growth (Table 1). These changes were produced by a rise in the fresh masses of the shoots and the roots, which increased progressively during plant growth. The content of protein gradually decreased during the progression of plant growth in both shoots and roots (Table 1).

Table 1. Fresh mass [g] and protein content [mg g^{-1}(f.m.)] in 3-d-old seedlings and in shoots and roots of 7 to 11-d-old pea plants. The data represent the means from at least three repetitions ± SE. Different letters indicate statistically significant differences according to Tukey's test ($P < 0.05$).

	Age [d]	Fresh mass	Protein content
Seedlings	3	0.302 ± 0.005d	6.119 ± 0.362b
Shoots	7	0.372 ± 0.009c	9.305 ± 0.555a
	9	0.513 ± 0.045b	8.014 ± 0.415a
	11	0.581 ± 0.006a	5.362 ± 0.423b
Roots	7	0.409 ± 0.007c	3.539 ± 0.163c
	9	0.505 ± 0.019b	2.558 ± 0.074d
	11	0.613 ± 0.010a	1.789 ± 0.338d

The glutathione contents were much higher in the shoots than in the roots. This difference was due to a higher level of both reduced (GSH) and oxidised (GSSG) glutathione in shoots than in roots (Table 2). However, the roots exhibited a higher redox state of glutathione than the shoots, particularly after 7 d of growth. A similar situation occurred for the ascorbate contents: the shoots showed higher content of total ascorbate than the roots after 11 d of growth. This difference was due to a higher content of reduced ascorbate. The shoots had a lower content of oxidised ascorbate (DHA) than the roots, giving rise to a higher redox state of ascorbate in shoots than in the roots, mainly after 9 and 11 d of growth (Table 2). It is worth noting that the 3-d-old seedlings had the highest DHA content, correlated with the highest AAO activity (Table 2). Similarly, DHA contents in roots from 9 and 11-d-old plants were much higher than in shoots, and this result also was paralleled by a greater AAO activity in relation to the activity values of the shoots (Table 2).

We also monitored the activities of antioxidant enzymes during the development of pea plants. In all cases, the activities of APX, MDHAR, DHAR and CAT were mostly higher in the shoots than in roots. Specific APX activity increased in the shoots up to 9 d of growth and further decreased in both shoots and roots. At 11 d of growth, APX activity in shoots was 3-times lower than in 7-d-old plants (Table 3). The CAT activity in shoots was 3- or 4-fold higher than in roots. However, the CAT activity found in 3-d-old seedlings was even lower than that in plant roots (Table 3). POX activities were considerably higher in roots than in shoots, and in roots a strong increase during the progression of growth was observed (Table 3).

The MDHAR activity was higher in young seedlings

Table 2. Reduced or oxidized glutathione and ascorbate contents [nmol g^{-1}(f.m.)], redox state of glutathione and ascorbate, and ascorbate oxidase (AAO) activity [nmol mg^{-1}(protein) min^{-1}] in 3-d-old seedlings and in shoots and roots of 7 to 11-d-old pea plants. The data represent the means ± SE from at least three repetitions. Different letters indicate statistically significant differences according to Tukey's test ($P < 0.05$).

	Age [d]	GSH	GSSG	GSH/(GSH+ GSSG)	ASC	DHA	ASC/(ASC+DHA)	AAO
Seedlings	3	105.9±1.3b	12.5±0.8bc	0.904±0.014bc	1075±10b	278± 7a	0.796±0.019c	564.3± 9.2a
Shoots	7	135.7±6.8a	18.4±2.1a	0.874±0.028c	1251±63b	31± 4d	0.947±0.016a	128.3± 6.5d
	9	137.9±8.4a	13.4±2.0ab	0.917±0.027ab	1181±51b	85± 7c	0.925±0.027ab	165.0±24.0c
	11	99.1±1.8b	11.4±0.9ab	0.907±0.017ab	1571±95a	89± 8c	0.960±0.018a	212.7±13.6b
Roots	7	74.0±3.5c	7.7±0.6cd	0.908±0.012ab	1176±15b	27± 4d	0.982±0.013a	542.3±15.5a
	9	85.0±6.0c	8.0±0.3cd	0.942±0.006a	1195±91b	157±10b	0.829±0.014c	583.7± 9.8a
	11	56.8±8.6d	3.8±0.33d	0.940±0.013a	1161±75b	172±15b	0.811±0.033c	542.4±11.5a

Table 3. Specific activities of antioxidant enzymes APX, MDHAR, DHAR GR [nmol mg^{-1}(prot.)], POX [µmol mg^{-1}(prot.)], CAT [mmol mg^{-1}(prot.)] and SOD [U mg^{-1}(prot.)] in 3-d-old seedlings and in shoots and roots of 7 to 11-d-old pea plants. The data represent the means ± SE from at least three repetitions. Different letters indicate statistically significant differences according to Tukey's test ($P < 0.05$). nd - not detected.

	Age [d]	APX	MDHAR	DHAR	GR	POX	CAT	SOD
Seedlings	3	49.1±2.2c	60.39±0.75a	1.03±0.28c	7.49±0.20b	496.7±16.2e	0.36±0.02d	51.80±6.2c
Shoots	7	72.7±2.9a	36.57±3.60c	1.88±0.14bc	8.44±0.58ab	328.9±27.7f	2.60±0.14a	36.40±1.5d
	9	65.8±3.5b	35.10±1.50c	2.01±0.23ab	5.81±0.50c	374.9±13.6f	2.17±0.10a	42.92±1.6d
	11	24.8±1.2d	34.14±1.39c	2.47±0.35a	5.98±0.45c	675.3±37.4d	2.04±0.15ab	56.71±1.5c
Roots	7	45.6±1.7c	44.34±1.66b	nd	5.54±0.15c	875.0±37.9c	0.65±0.02cd	53.24±2.7c
	9	14.7±0.41e	48.47±1.57b	nd	8.45±0.28b	2329.0±74.3b	0.51±0.01d	89.51±2.9b
	11	2.5±0.1f	34.10±1.97c	nd	8.86±0.10a	4151.0±83.9a	0.73±0.01c	196.46±1.9a

than in older plants. This activity did not show important changes in the shoots during further growth, but the values were lower than in roots. Nevertheless, in roots a decrease in MDHAR activity was noticed at the 11th day (Table 3). DHAR activity was detected only in the shoots and an increase was also observed during the growth of pea plants. The DHAR activity found in 3-d-old seedlings was 2 times lower to that observed in shoots and a time-dependent increase in specific DHAR activity was observed. It is noticeable that, in this pea cultivar, the activity of MDHAR was much higher than that of DHAR; indeed, no DHAR activity was detected in roots.

In shoots, GR decreased with the progression of growth, whereas in roots an increase was noticed at the 9th and 11th day, in relation to plants sampled at 7th day (Table 4). Finally, superoxide dismutase (SOD) activity increased in shoots during the progression of growth, and at 11th day values were similar to those found in young seedlings (Table 4). In root, specific SOD activity was higher than in shoots and a age-dependent increase was found.

Discussion

The results show the changes in the antioxidative metabolism during the development of both the shoots and the roots of pea plants. It has been reported that, besides plant hormones, ASC availability also affects vegetative development (De Gara 2003). Treatments increasing the cellular ASC content promote cell elongation, with a consequent increase in plant growth (Arrigoni *et al.* 1997, Córdoba and González-Reyes 1994). Different mechanisms have been proposed to explain this stimulatory effect of ASC: *1*) in the presence of traces of Cu^{2+} or H_2O_2, apoplastic ASC triggers hydroxyl radical generation that causes the non-enzymatic scission of the polysaccharides matrix; this promotes wall loosening and seems to be involved in the control of both elongation growth and fruit softening (Schopfer 2001); *2*) ASC is required for the synthesis of oxalate, the presence of which in the apoplast reduces the Ca^{2+}-dependent pectin immobilisation, by sequestering calcium ions and reducing wall stiffening (Davey *et al.* 2002), and *3*) ASC could enhance cell wall plasticity by affecting the activity of the POXs responsible for cell wall stiffening (De Gara 2003).

At 11th day, the growth of pea shoot was correlated positively with the ASC content, but negatively with GSH content. However, at 7th and 9th day, the inverse situation occurred. DHA contents were much higher in the youngest seedlings than in the other periods of growth analysed. This result can be explained by a high AAO activity shown at this stage of growth. Also, the higher DHA content in the roots, compared with the shoots, was correlated with a high root AAO activity. The DHA accumulation brought about a decrease in the redox state of ascorbate, in young seedlings as well as in roots. Paradoxically, specific MDHAR activity was higher in young seedlings and in root than in shoots, although in a fresh mass base, MDHAR was higher in shoots than in roots (data not shown). This difference can explain partially the lower contents of DHA as well as the higher redox state of ascorbate found in shoots compared with roots. On the other hand, only in the shoots of the plants or in the young seedlings could DHAR activity be measured, it being undetectable in the roots. This can imply that differences in DHA between shoots and roots can be explained partially by DHAR activity. Both MDHAR and DHAR participate in ASC regeneration, by using, respectively, NADH or GSH as reducing power (Noctor and Foyer 1998). In the present work, MDHAR activity was much higher than DHAR activity. These data suggest that, the ASC in pea is recycled mainly *via* NADH in shoots, and exclusively in roots. These data are in agreement with previous results obtained for the soluble fraction and chloroplasts from mature plants of pea cv. Alaska grown in hydroponic cultures, showing MDHAR activity values up to 4-fold higher than those of DHAR (Díaz-Vivancos *et al.* 2008). So, both the lower AAO and higher DHAR activities can explain the lower contents of DHA measured in the shoots of pea plants, relative to the roots and to the data obtained in 3-d-old seedlings. In tobacco BY-2 cells, mRNA levels of AAO were correlated positively with the DHA content (Kato and

Esaka 1999). However, other possibilities can not be rule out, such as the thioredoxin-thioredoxin reductase system (De Gara 2003).

It appears that AAO may be involved in plant cell growth, its role during the early phase of plant growth being more important. AAO regulates ASC/DHA levels during the cell cycle, even in cultured cells (Kato and Esaka 1999). High AAO activities are known to be associated with periods of rapid cell expansion during plant growth and development (Kato and Esaka 1999). Our data agree with the previous findings: AAO activity was greater in young seedlings than in plants shoots. It has been demonstrated that AAO regulates the apoplastic ASC pool and therefore the redox state of ascorbate (ASC/ASC + DHA) (Pignocchi *et al.* 2003). The activity of apoplastic AAO also seems to be positively correlated with cell elongation (Kato and Esaka 1999). Our results agree with the data described in the literature that the redox state of ascorbate plays a role in plant growth (Córdoba and Gónzalez-Reyes 1994, Arrigoni *et al.* 1997, De Pinto and De Gara 2004).

In the present work, the redox state of glutathione was higher in roots than in shoots, at least in 7-d-old plants. However, GSH contents were much higher in shoots than in roots and this result was not in correspondence with higher specific GR activities. Only at 7th day GR activity was statistically higher in shoots than in roots, but not in the other periods. When data were expressed in a fresh mass basis, GSH contents of shoots correlated with a GR activity (data not shown). GR is involved in GSH recycling, by using NADPH as an electron donor (Noctor and Foyer 1998), and increased GR activity, besides the biosynthesis rate, could account for the higher GSH content found in the shoots of pea plants compared with the roots. Several reports suggest that GSH plays a key role in promoting signals that permit cell cycle progression, increasing root growth (Vernoux *et al.* 2000, Maughan and Foyer 2006). It has been reported that GSH accumulates in the root tips, suggesting that actively-growing root tissues have a requirement for GSH (Maughan and Foyer 2006). Depletion of intracellular GSH, by BSO treatment, inhibits post-embryonic root growth in *Arabidopsis* and tobacco (Vernoux *et al.* 2000). Moreover, in *Arabidopsis rml1* mutants, containing only 2.7 % of the extractable GSH, root tips did not divide after germination (Vernoux *et al.* 2000). Interestingly, *rml1* mutants could be rescued by GSH application to the growth medium. However, the mutant could not be rescued with GSSG, ascorbate or DTT (Vernoux *et al.* 2000).

Activities of the antioxidant enzymes were also recorded, and changes in H_2O_2-scavenging enzymes, such as APX and POX, were the most significant. The meristematic cells have a higher APX activity, both in the apoplastic space and the cytosol, than the differentiated cells; whereas during differentiation, POXs increase both the activity and the number of isoenzymatic forms which are present (De Pinto and De Gara 2004). This response was also observed in pea plants, where a progressive decrease in APX was observed, both in shoots and roots. It has been described that a decrease in apoplastic APX activity occurring during differentiation could render H_2O_2 available for POX activity allowing the processes of wall stiffening (De Pinto and De Gara 2004). Interestingly, the extracellular fluid of mature pea leaves does not shown APX activity (Hérnandez *et al.* 2001), and treatments that increased ASC contents and APX activity decreased POX activity in elongating onion roots (De Tulio *et al.* 1999, De Gara 2003).

A positive correlation between POX activity and plant growth was observed in pea shoots and roots. There are several lines of evidence indicating that plant POXs are involved in cell wall metabolism and development. In particular, POXs are responsible for stiffening of the cell wall and lignin and suberin deposition (De Gara 2003, Pomar *et al.* 2002). Enhanced POX activity in germinating wheat seeds can be considered to be a reflection of the enhancement of the growth and development of the embryo and seedlings (Cakmak *et al.* 1993). The over-expression of horse radish POX stimulated the growth of tobacco and hybrid aspen plants (Kawaoka *et al.* 2003). There are three possible explanations concerning the role of POXs in plant growth (Kawaoka *et al.* 2003). The first is the role of POXs in auxin catabolism: POX oxidises indolacetic acid, and the ratio of auxin to cytokinin is thought to be important for plant growth and development (Romano *et al.* 1991). Secondly, POX activity is associated with the lignification (Pomar *et al.* 2002). Finally, class III POXs have broad substrate specificity, and they can use ASC as an electron donor (Kvaratskhelia *et al.* 1997). Both AAO and class III POXs can act also as an ASC-removal system in the apoplastic space in order to facilitate the role of POXs in cell wall differentiation during plant development. It must be mentioned that ASC itself negatively affects the peroxidase reaction, which is important for wall stiffening and, in general, for cell wall differentiation (De Pinto and De Gara 2004, Ros Barceló *et al.* 2006). A negative correlation between ASC and POX has been reported: when an increase in ASC is experimentally induced in plant tissues, a decrease in POX activity also occurred. Moreover, in developing organs, cell or apoplastic fluid having high ASC contents has a low POX activity (Cordoba-Pedregosa *et al.* 2003, De Gara 2003). In our work, however, a positive correlation between DHA contents and POX activity was observed, mainly in roots (9 and 11 d of growth). In other words, we observed a correlation between an increase in POX activity and a decrease in the redox state of ascorbate.

SOD activity showed a time-dependent increase in pea plants, which could suggest an increased production of $O_2^{\cdot -}$. The increase in SOD in roots and shoots was parallel with a rise in POX activity, but not with the other H_2O_2-scavenging enzymes, CAT or APX. Several studies identified roles for ROS in plant development. For example, $O_2^{\cdot -}$ was found to be essential for morphogenesis in etiolated wheat seedlings. Furthermore, seedlings incubated with antioxidants had reduced $O_2^{\cdot -}$ and disturbed development (Shorning *et al.* 2000). Several authors described that H_2O_2 is necessary for leaf or root elongation (Rodriguez *et al.* 2002, Córdoba-Pedregosa *et al.* 2003).

Data showed changes in the antioxidative metabolism during development of pea plants. Results suggest that changes in redox state of ascorbate and glutathione as well as the regulation in ROS production could acts as signalling stimuli during plant growth.

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